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Original Article

EVALUATION OF WETTABLE POWDER FORMULATIONS OF *NOMURAEA RILEYI* (FARLOW) SAMSON AGAINST 3RD INSTAR OF *SPODOPTERA LITURA* UNDER LABORATORY CONDITIONS AT DIFFERENT STORAGE INTERVALS

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ABSTRACT

The present study was undertaken to emphasize the effect of some Wettable powder formulations of an entomopathogenic fungi i.e., Nomuraea rileyi (Order: Hypocreales, Family: Clavicipitaceae), against tobacco cut worm in a lab experiment which was conducted during 2013-14, at S.V Agricultural college, Tirupati. Its six different formulations viz. carrier based Wettable powder formulations, were prepared and stored at two storage conditions i.e. at 4°C and 25°C.These formulations were bioassayed against 3rd instar of Spodoptera litura (Order: Lepidoptera, Family: Noctuidae) after making four different concentrations i.e., 1×10^8 spores ml^{-1} to 1×10^5spores ml^{-1} by using serial dilution technique at 30 days interval upto 90 days of storage. Talc, Starch and Rice flour formulations were observed to be most effective as determined by measuring larval mortality as well as ease of applicability. The study also revealed that with increase in storage period and storage temperature conditions the larval mortality is decreasing gradually, Whereas with increase in concentration of the fungal formulation, the larval mortalities are also increasing.

KEYWORDS: N. Rileyi, Wettable Powder Formulations, S. Litura, Days After Storage & Mortality

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INTRODUCTION

After realizing the negative impacts (*i.e.,* pollution, pesticide residue, pesticide resistance *etc,*) of tremendous usage of pesticides on nature and natural resources, shifting of focus towards production of more reliable, more stable and ecofriendly microbial control agents was being observed during now a days. Entomopathogens like virus, bacteria, fungi and nematodes produced artificially and formulated into microbial biopesticides, can provide best alternatives to chemicals. Among the different entomopathogens, Entomopathogenic fungi are often reported as causing high levels of mortality (epizootics) in nature and they are highly virulent. Among all Entomopathogenic fungi, *Nomuraea rileyi* is a potential, biocontrol agent. More than 30 species of lepidopterous insect pests were listed as susceptible to *N. rileyi* (Ignoffo,1981) and it is responsible for causing natural mortality in as many as 51 lepidopteran insects throughout the world (Lingappa and Patil, 2002). Noctuid Caterpillars are found as most sensitive hosts for *N. rileyi*. Vimala Devi *et al.* (2000) reported, cost effective and rapid multiplication of *N. rileyi*. Quite a attempt have been made to prepare formulations of the fungus *N. rileyi* by using six different carrier materials, in order to evaluate the efficacy of prepared wettable

powder formulations of *N. rileyi* under laboratory conditions, on one of the most problematic lepidopteran pest *i.e., Spodoptera litura, which was responsible for causing more economic losses in several crops* (Rao *et al.,* 1993).

MATERIALS AND METHODS

In order to prepare Wettable powder formulations (WP) of *N. rileyi* the protocol of Swetha (2011) was followed. WP formulations were prepared by using six inert materials *i.e.,* Talc, starch, rice flour, jowar flour, wheat flour and ragi flour. Hundred gm of each carrier was taken in 250 ml conical flasks and placed in an oven at 160°C for 1 hour on each day for 2 days until for sterilization. After sterilization, the carriers were mixed each with 2.5 gm of harvested *N. rileyi* spores under aseptic conditions. Two to three drops of tween-20 was added to the mixture as wetting agent for uniform mixing of spores with carriers. Each material was separated into 2 halves. One half was stored in refrigerator at 4 ºC and another in incubator at 25ºC. The following are the formulations made by using spore mass (WP formulations) *i.e.,* Talc formulation, starch formulation, rice flour formulation, wheat flour formulation, jowar flour formulation and ragi flour formulation.

The test insect *S. litura* was maintained on natural feed *i.e.,* on castor leaves throughout the lab experiment for conducting virulence studies at 30 days interval upto 90 days. Spore suspensions of 1×10^8 to 1×10^5spores ml^{-1} concentrations of all the six wettable powder formulations were prepared. Initially stock suspensions (*i.e.,* 1×10^8 spores ml^{-1} was prepared after dissolving the 0.5 g of each wettable powder formulation in 100 ml distilled water and filtered through muslin clothe, by adjusting the spore count of *N. rileyi* with the help of Neubauer haemocytometer. From these stock suspensions, serial dilution of 1×10^7 to 1×10^5 spores ml^{-1} were prepared. All the four concentrations of each six (Talc, starch, rice flour, wheat flour, jowar flour and ragi flour) wettable powder formulations of *N. rileyi* were applied to 3rd instar larvae with the help of hand automizer (Swetha, 2011) by using leaf application method. For Ten *S. litura* larvae were used for each treatment and they were allowed to crawl on the treated castor leaves for the first day and from next day onwards, fresh castor leaves were supplied to the larvae. Daily observations were recorded on larval mortalities. The larval mortality due to formulations of *N. rileyi* was expressed as per cent mortality using the formula.

$$\text{Per cent mortality} = \frac{\text{Number of larvae dead} \times 100}{\text{Total number treated}}$$

RESULTS AND DISCUSSIONS

Larval Mortalities of 3rd Instar of *S. Litura* with Wettable Powder Formulations of *N. Rileyi*

At Refrigerated Conditions (At 4 °C)

- **Thirty Days after Storage (30 DAS):** No significant difference was found between starch formulation of *N. riley*i with spore suspension of 1×10^8 and 1×10^7 spores ml^{-1} with respect to larval mortality of 3rd instar larvae of *S. litura.*
- **Sixty Days after Storage (60 DAS):** All the concentrations of talc, starch and rice flour formulations recorded above 50 per cent larval mortality where as wheat, jowar and ragi formulations recorded upto 36 per cent of larval mortality with their four different concentrations (Table 1.)
- **Ninety Days after Storage (90 DAS):** Talc, starch and rice flour formulations gave upto 50 per cent mortality where as wheat, jowar and ragi flours retained virulence upto 26 per cent with their all concentrations.

Formulations Stored at 25 °C

- **Thirty Days after Storage (30 DAS):** Third instar larvae were became susceptible from 33 to 60 per cent with four different concentrations of WP formulations of *N. rileyi* at the end of one month of storage.
- **Sixty Days after Storage (60 DAS):** 36.93 per cent mean larval mortality was obtained after 60days of storage. Up to 40 per cent of larval mortality was obtained with all the concentrations of talc and starch formulations after 2 months of storage. There is no significant difference between $1{\times}10^8$ and $1{\times}10^7$ spores ml^{-1} concentrations of ragi flour formulation with regard to virulence of *N. rileyi* against 3rd instar larvae of *S. litura* (Table 1).
- **Ninety Days after Storage (90 DAS):** The mean larval mortality recorded was 24.13. Only talc formulation recorded above 35 per cent larval mortality with all four different spore concentrations. In remaining formulations, 10 to 37 per cent mortality was noticed.

In the present study, talc was found superior by recording 60 per cent of larval mortality at 30 DAS, 50 per cent at 60 DAS and 47 per cent larval mortality at the end of 90 days of storage with higher concentration of $1{\times}10^8$ spores ml^{-1}. Second and third positions in holding the *N. rileyi* in pathogenic condition were occupied by starch (70 per cent at 30 DAS, 57 per cent at 60 DAS and 37 per cent after 3 months of storage) and rice flour (63 per cent at 30DAS, 47 per cent at 60 DAS and 27 per cent after 3 months of storage) respectively. The ragi flour was found to be inferior by showing least mortality.

The superiority of talc and starch in maintaining the viability and virulence of *N. rileyi* may be primarily due to their good amount of carbohydrate content and mineral composition. Talc contains 35-100 per cent carbohydrate and starch having 91 per cent. Talc is a organic carrier, which enhances the survival rate and growth of organism by protecting it from desiccation and death of cells. Talc has good storage stability, miscibility with water and convenient for application through conventional spraying equipment (Brar *et al*., 2006). It is used as a carrier for formulation and development due to its inert nature and easy availability as raw material from soapstone industries (http:// www. Luzenac.com/food.htm). Similar findings were recorded when Swetha (2011) evaluated some wettable powders of *N. rileyi* against the 3rd instar, in a lab experiment in which Talc powder formulations of *N. rileyi* recorded 45 per cent mortality of *S. litura* larvae followed by corn flour 32.50 after 90 days of storage.

CONCLUSIONS

When Mallikarjuna *et al.* (2010) formulated *N. rileyi* as wettable powder by using different carrier materials (*viz.,* bentonite + glucose (7:1) and bentonite + sucrose (7:1), talc + glucose (7:1) and talc + sucrose (7:1)) and evaluated the formulations against two important noctuid pests, *Spodoptera litura* and *Helicoverpa armigera*. The mortalities obtained were in the range of 72-87 per cent in case of *S. litura*, whereas 66 - 88 per cent in case of *H. armigera*.

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APPENDICES

Table 1: Virulence of Wettable Powder Formulations of *N. rileyi* against 3rd Instar Larvae of *S. litura*

Refrigerator Stored Wettable Powder Formulations (at 4°C)						Incubator Stored Wettable Powder Formulations (at 25°C)		
S.No.		**Treatments**	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**
1	Talc	1x10^{8} spores ml^{-1}	63.33 ^{abc} (52.78)	56.67 ^{ab} (48.85)	50.00 ^{a} (45.00)	60.00 ^{bcd} (50.74)	50.00 ^{abc} (44.98)	46.67 ^{a} (43.06)
		1x10^{7} spores ml^{-1}	60.00 ^{bcd} (50.77)	53.33 ^{abc} (46.92)	50.00 ^{a} (45.00)	56.67 ^{cde} (48.82)	46.67 ^{bcd} (43.06)	43.33 ^{ab} (41.13)
		1x10^{6}spores ml^{-1}	56.67 ^{cde} (48.85)	50.00 ^{bcd} (45.00)	46.67 ^{ab} (43.08)	53.33 ^{def} (46.90)	43.33 ^{cde} (41.13)	40.00 ^{abc} (39.21)
		1x10^{5}spores ml^{-1}	53.33 ^{def} (46.92)	46.67 ^{cde} (43.08)	40.00 ^{bcd} (39.23)	50.00 ^{efg} (44.98)	40.00 ^{def} (39.21)	36.67 ^{bcd} (37.21)
2	Starch	1x10^{8} spores ml^{-1}	70.00 ^{a} (56.79)	60.00 ^{a} (50.77)	43.33 ^{abc} 41.15)	70.00 ^{a} (56.76)	56.67 ^{a} (48.82)	36.67 ^{bcd} (37.21)
		1x10^{7} spores ml^{-1}	70.00 ^{a} (56.79)	56.67 ^{ab} (48.85)	40.00 ^{bcd} (39.23)	66.67 ^{ab} (54.76)	53.33 ^{ab} (46.90)	33.33 ^{cde} (35.20)
		1x10^{6}spores ml^{-1}	63.33 ^{abc} (52.78)	53.33 ^{abc} (46.92)	36.67 ^{cde} (37.22)	63.33 ^{abc} (52.75)	50.00 ^{abc} (44.98)	30.00 ^{def} (30.98)
		1x10^{5}spores ml^{-1}	60.00 ^{bcd} (50.77)	53.33 ^{abc} (46.92)	36.67 ^{cde} (37.22)	60.00 ^{bcd} (52.75)	46.67 ^{bcd} (43.06)	26.67 ^{efg} (30.98)
3	Rice Flour	1x10^{8} spores ml^{-1}	66.67 ^{ab} (54.78)	56.67 ^{ab} (48.85)	40.00 ^{bcd} (39.23)	63.33 ^{abc} (52.75)	46.67 ^{bcd} (43.06)	26.67 ^{efg} (30.98)
		1x10^{7} spores ml^{-1}	63.33 ^{abc} (52.78)	53.33 ^{abc} (46.92)	40.00 ^{bcd} (39.23)	60.00 ^{bcd} (50.74)	43.33 ^{cde} (41.13)	23.33 ^{fgh} (28.76)
		1x10^{6}spores ml^{-1}	60.00 ^{bcd} (50.77)	50.00 ^{bcd} (45.00)	36.67 ^{cde} (37.22)	56.67 ^{cde} (48.82)	40.00 ^{def} (39.21)	23.33 ^{fgh} (28.76)
		1x10^{5}spores ml^{-1}	56.67 ^{cde} (48.85)	46.67 ^{cde} (43.08)	33.33 ^{def} (35.22)	53.33 ^{def} (46.90)	36.67 ^{efg} (37.21)	20.00 ^{ghi} (26.55)
4	Wheat Flour	1x10^{8} spores ml^{-1}	50.00 ^{efg} (45.000	46.67 ^{cde} (43.08)	36.67 ^{cde} (37.22)	50.00 ^{efg} (44.98)	36.67 ^{efg} (37.21)	20.00 ^{ghi} (26.06)
		1x10^{7} spores ml^{-1}	46.67 ^{fgh} (43.08)	43.33 ^{def} (41.15)	33.33 ^{def} (35.22)	46.67 ^{fgh} (43.06)	33.33 ^{fgh} (35.20)	16.67 ^{hij} (23.86)
		1x10^{6}spores ml^{-1}	43.33 ^{ghi} (41.15)	40.00 ^{efg} (39.23)	33.33 ^{def} (35.22)	43.33 ^{ghi} (41.13)	30.00 ^{ghi} (32.98)	16.67 ^{hij} (23.84)
		1x10^{5}spores ml^{-1}	40.00 ^{hi} (39.23)	36.67 ^{fgh} (37.22)	30.00 ^{efg} (33.21)	40.00 ^{hij} (39.21)	26.67 ^{hi} (30.98)	13.33 ^{ij} (21.13)
5.	Jowar Flour	1x10^{8} spores ml^{-1}	46.67 ^{fgh} (43.08)	36.67 ^{fgh} (37.22)	26.67 ^{fgh} (31.00)	43.33 ^{ghi} (41.13)	33.33 ^{fgh} (35.20)	23.33 ^{fgh} (28.76)
		1x10^{7} spores ml^{-1}	43.33 ^{ghi} (41.15)	33.33 ^{ghi} (35.22)	26.67 ^{fgh} (31.00)	40.00 ^{hij} (39.21)	30.00 ^{ghi} (33.19)	20.00 ^{ghi} (26.53)
		1x10^{6}spores ml^{-1}	40.00 ^{hi}	30.00 ^{hi}	23.33 ^{ghi}	36.67 ^{ij}	30.00 ^{ghi}	16.67 ^{hij}

			(39.23)	(33.21)	(28.78)	(37.21)	(32.98)	(23.84)
		1x10^{5}spores ml^{-1}	36.67^{i} (37.22)	26.67^{i} (31.00)	20.00^{hi} (26.57)	33.33^{j} (35.20)	23.33^{i} (28.76)	13.33^{ij} (21.13)
6	Ragi Flour	Ragi 1x10^{8} spores ml^{-1}	50.00^{efg} (45.00)	33.33^{ghi} (35.22)	26.67^{fgh} (31.00)	43.33^{ghi} (41.13)	30.00^{ghi} (33.19)	20.00^{ghi} (26.55)
		1x10^{7} spores ml^{-1}	46.67^{fgh} (43.08)	33.33^{ghi} (35.22)	23.33^{ghi} (28.78)	43.33^{hij} (41.13)	30.00^{ghi} (33.19)	16.67^{hij} (23.84)
		1x10^{6}spores ml^{-1}	43.33^{ghi} (41.15)	30.00^{hi} (33.21)	20.00^{hi} (26.57)	36.67^{ij} (37.21)	26.67^{hi} (30.98)	13.33^{ij} (21.13)
		1x10^{5}spores ml^{-1}	36.67^{i} (37.22)	26.67^{i} (31.00)	16.67^{i} (23.86)	33.33^{j} (35.20)	23.33^{i} (28.76)	10.00^{i} (18.42)
		Untreated check	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
		General mean	50.67 (44.76)	42.13 (39.72)	32.40 (33.85)	48.53 (43.51)	36.93 (36.65)	24.13 (28.43)
		SE(m)	1.51	1.68	1.64	1.52	2.07	1.99
		C.D.(0.05)	4.30	4.77	4.66	4.32	5.87	5.67

- **The values are means of three replications.**
- **Figures in the parentheses are angular transformed values.**
- **Mean followed by same letter in the column do not differ significantly by DMRT (p = 0. 0 1)**